



N-RAS lipid anchor adsorption to membranes as a function of lipid composition and curvature

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prostate cancer cells showed that exosomes are particularly enriched in long-chain SM (*Biochim. Biophys. Acta*, 2013, 1831, 1302–1309). In this study, we use atomistic molecular dynamics simulations to consider asymmetric lipid membrane models whose compositions are based on these data. The simulations show interdigitation of the long-chain SM to the opposite leaflet, which is quite expected, but interestingly we found out that interdigitation was particularly strong in asymmetric bilayers compared to symmetric ones. We observed that the conformational order of the amide-linked SM chain increases the deeper it penetrates to the opposing leaflet. We further showed that cholesterol modulates the effect of SM interdigitation by influencing the conformational order of lipid hydrocarbon chains in the opposing (cytosolic) leaflet.

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Transbilayer Registration of Liquid-Ordered Domains: No Interactions at the Membrane Midplane Required

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The mechanism responsible for the registration of liquid-ordered (Lo) domains in the two membrane leaflets is a matter of debate. As an alternative to the thus far enigmatic interactions at the membrane midplane, we propose that minimization of the line tension around the thicker Lo bilayer drives registration. Based on the continuum elasticity theory, we demonstrate that the thickness mismatch at the Lo/Ld boundary results in elastic deformations of lipid molecules. The deformations require a minimum of energy F_D when the domain boundaries are shifted relative to each other by several nanometers¹. Accumulation of lipids or peptides with non-zero spontaneous curvature in the thin rim around the domains further sharply decreases F_D , thus explaining the line activity of monosialoganglioside GM1. In addition to F_D , the mutual attraction of stiffed membrane regions must contribute to domain registration². Since energy F_U is required to prevent membranes from undulating, maximizing the membrane area that is free to undulate by aligning the stiff Lo domains from opposing leaflets minimizes F_U . Thus, domain registration minimizes F_U and F_D explaining why (i) transbilayer coupling can occur without interaction at the membrane midplane and (ii) registration does not require specific lipids in Lo domains.

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1) Galimzyanov, Molotkovsky, Bozdaganyan, Cohen, Pohl, Akimov. Elastic Membrane Deformations Govern Interleaflet Coupling of Lipid-Ordered Domains. *Phys.Rev.Lett.* 115:088101, 2015.

2) Horner, Antonenko, Pohl. Coupled diffusion of peripherally bound peptides along the outer and inner membrane leaflets. *Biophys.J.* 96:2689-2695, 2009.

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N-RAS Lipid Anchor Adsorption to Membranes as a Function of Lipid Composition and Curvature

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Protein recruitment to biological membranes is motivated by either highly selective recognition of specific target membrane components or non-specific attraction to general physical properties of the membrane, such as charge, lipid heterogeneity, and curvature. Here we discuss the interaction between lipid-anchored proteins and lipid membranes from a comprehensive examination of how features of the membrane and its lipid constituents, including lipid head-group size, composition, heterogeneity, membrane thickness, degree of unsaturation, and membrane geometry, effect the adsorption ability of the proteins. Of key importance is the strong interconnection among these compositional and morphological elements in mediating the binding of peripheral membrane proteins. As a model protein, we use the dual lipidated (palmitoyl and farnesyl) anchoring motif of the signaling GTPase N-Ras (tN-Ras). We find marked augmentation in tN-Ras adsorption with increasing degree of membrane curvature—a trend that is tightly regulated by the bilayer characteristics mentioned above. Experimental results are fully reproduced by a

molecular level theoretical model of the systems under study. Of note, the theory suggests an explicit dependence on the lateral pressure profile of the membrane's hydrophobic region to be the mechanism and cause of variation in protein density with membrane curvature and composition. Relief in the lateral pressure of the bilayer's outer leaf, upon its expansion induced by increasing curvature, reduces the work requirement for lipid-anchor insertion into the membrane. Furthermore, the inherent pressure profile of the hydrophobic channel, at a given curvature, is unique with regard to membrane composition, which allows for fine-tuning of lipidated-protein density.

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Segmentation of Membrane Protein Motion in the Axon Initial Segment

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The axon initial segment (AIS) is a structure rich in specific cytoskeletal molecules that play important roles in the concentration of ion-channels that are required for action-potential generation. The establishment of a postulated diffusion barrier to the lateral exchange of membrane molecules in the AIS correlates with the enrichment of specific cytoskeletal molecules at this structure during development.

Recently, a repetitive pattern of actin, spectrin and ankyrin forming ring-like structures perpendicular to the direction of axonal propagation has been discovered, that is interconnected via spectrin tetramers. This structure may finally provide the long sought direct physical correlate to the diffusion barrier at the AIS.

Here, we perform repeated high-throughput single-molecule tracking on individual live primary hippocampal neurons during AIS development (DIV 3 - 10). We furthermore analyze the lateral mobility of lipid-anchored and transmembrane molecules with microsecond tracking at a resolution of few nanometers via interferometric scattering (iSCAT). Finally, we correlate the lateral motion of membrane molecules to the organization of the AIS cytoskeleton.

We find that the lateral motion of membrane molecules becomes reduced in the AIS during development and that this reduction correlates with cytoskeletal organization into ring-like structures. The lateral motion of membrane molecules in the AIS plasma membrane is locally confined to a within a repetitive pattern of 190 nm spaced segments along the AIS axis, consistent with the observed spacing of the cytoskeletal rings.

Our data provide mechanistic insight into the diffusion barrier function in of the AIS.

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Mechanistic Insights into Membrane Bending by Protein Crowding: Understanding the Role of Membrane Composition, Phase Separation and Free Energy of Protein Binding

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Morphological changes in lipid membranes are hallmarks of a number of cellular processes like sorting, transport, etc. The dense crowding of the membrane environment with proteins and receptors has motivated many thorough academic investigations into the effects of macromolecular crowding on membrane surfaces. A number of recent studies have indicated that membrane reshaping could be driven by steric pressure between proteins co-localized on membrane domains. While these studies provide conclusive evidence for the membrane bending process, a detailed physical and mechanistic basis for this phenomenon is lacking. We provide a thermodynamic picture for this phenomenon through Isothermal Titration Calorimetry (ITC), Differential Scanning Calorimetry (DSC) and fluorescence microscopy using Ni-Nitrilotriacetic (NTA) acid and His-Tag interaction as a model system. Using ITC, we observe almost an order of magnitude increase in binding affinity for NTA-functionalized liposomes that display gel-fluid phase coexistence, as opposed to homogenous fluid compositions. This elevated affinity could be eliminated by thermal phase transition from gel-fluid to fluid, highlighting the importance of phase separation in modulating the strength of this binding interaction. DSC revealed that protein binding modulates the long-range lipid order substantially. In conjunction with the complicated nature of the binding isotherm, the DSC results indicate that the protein-binding event is coupled to a secondary exothermic process, presumably due to membrane deformation. Further ITC and fluorescence microscopy experiments reveal that the formation of